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# A Bicyclic Cispentacin Derivative as a Novel Reverse Turn Inducer in a GnRH Mimetic

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A cispentacin-derived bicyclic $\beta$-amino acid (Bic) has been synthesized and incorporated into the 6-position of GnRH. The new GnRH analogue has been characterized with respect to its structure in solution and its activity and affinity toward the human GnRH receptor.

The synthesis and application of rigid amino acid templates to generate peptidomimetics of various kinds is a research area of ever-increasing volume and importance in bioorganic chemistry.[1] Most of these templates are derived from $\alpha$-amino acids, in particular, proline derivatives,[2] and have been introduced into peptides in order to induce the formation of $\beta$-turn-like geometries.[3]

Usually, incorporation of $\beta$-amino acids results in enhanced stability against enzymatic cleavage, but at the expense of lower biological activity. Therefore, considerably less is known about the conformational preferences of linear peptides containing one or more $\beta$-amino acids as turn inducers.[4] To get more insight into the potential of such an approach, we have synthesized a cispentacin[5]-like unnatural rigid bicyclic $\beta$-amino acid, called Bic (**1**).
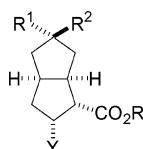
1a: R = H; R$^1$,R$^2$ = -O-CH$_2$-C(CH$_3$)$_2$-CH$_2$-O-; Y = NHFmoc

1b: R = Me; R$^1$,R$^2$ = -O-CH$_2$-C(CH$_3$)$_2$-CH$_2$-O-; Y = NHBoc

1c: R, R$^1$ = H, R$^2$ = OH, Y = NH$_2$

The Bic-core provides a rigid template for a variety of different alignments of carboxyl, amino, and side-chain



**FIGURE 1.** X-ray crystal structure of Boc-protected $\beta$-amino ester **1b** (racemic material, enantiomer of **1b** shown).

functionalities. Owing to the pseudoaxial and -equatorial arrangement of the appendages, defined turn angles can be obtained as demonstrated in the graphical representation of the X-ray structure of Boc-protected $\beta$-amino ester **1b** (Figure 1). The distance between the ester-carbonyl-C (the later C-terminus) and the Boc-carbamate nitrogen (the later N-terminus) is 2.90 Å, and the dihedral angle of the appendages around the Bic core is 39°, which is reflected in the peptide turn structure later.

As the $\beta$-hydrogen of $\beta$-amino acids is not acidic and the carboxyl function is in a thermodynamically favored exo arrangement, epimerization at these centers, which is a major concern in peptide synthesis with $\alpha$-amino acids, is not problematic for **1**.

Fmoc-Bic(Ketal)-OH[6] (**1a**) was synthesized in multigram amounts from enantiomerically pure $\beta$-hydroxy ester **2**[7] in five steps and 65% overall yield (Scheme 1).

For the introduction of the $\beta$-amino group, a conversion of $\beta$-hydroxy ester **2** into the corresponding nosylate, substitution by azide, and reduction with H$_2$ and Pd/C was found to be the most effective and convenient

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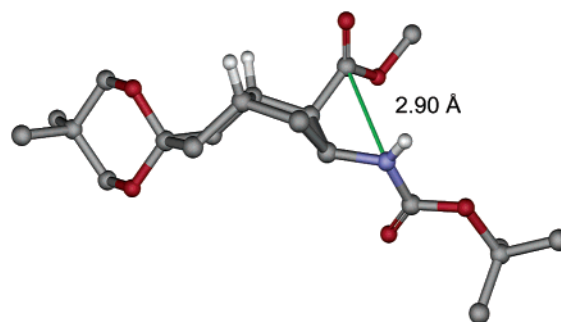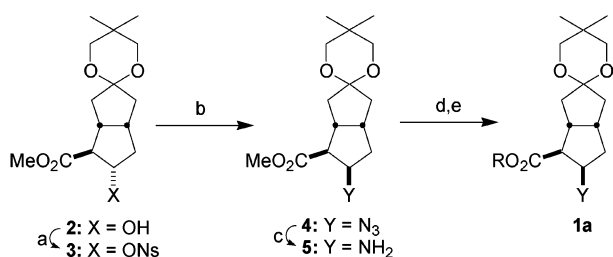
† Universität Frankfurt.

(1) Hanessian, S.; McNaughton-Smith, G.; Lombart, H.-G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789−12854 and references therein. See also Mulzer, J.; Schülzchen, F.; Bats, J.-W. *Tetrahedron* **2000**, *56*, 4289−4298.

(2) Baures, P. W.; Ojala, W. H.; Gleason, W. B.; Johnson, R. L. *J. Peptide Res.* **1997**, *50*, 1−13.

(3) (a) Nagai, U.; Sato, K.; Nakamura, R.; Kato, R. *Tetrahedron* **1993**, *47*, 17, 3577−3592. (b) Mueller, R.; Revesz, L. *Tetrahedron Lett.* **1994**, *35*, 4091−4092.

(4) See, for examples: (a) Hibbs, D. E.; Hursthouse, M. B.; Jones, I. G.; Jones, W.; Abdul Malik, K. M.; North, M. *J. Org. Chem.* **1998**, *63*, 1496−1504 and references therein. (b) Jones, I. G.; Jones, W.; North, M. *J. Org. Chem.* **1998**, *63*, 1505−1513 and references therein.

(5) Cispentacin = 2-aminocyclopentanecarboxylic acid, 2-ACPC.

(6) Nomenclature and abbreviations for amino acid residues follow the recommendations of the IUPAC−IUB Commission on Biochemical Nomenclature (*Biochemistry* **1970**, *9*, 3471−3479 and http://www.chem.qmw.ac.uk/iupac/AminoAcid/).

(7) Petzold, K.; Dahl, H.; Skuballa, W.; Gottwald, M. *Liebigs Ann. Chem.* **1990**, 1087−1091.

**SCHEME 1. Synthesis of Fmoc-Protected Bic 1a***$^a$*



2: X = OH
3: X = ONs
4: Y = N$_3$
5: Y = NH$_2$
1a

*$^a$* Key: (a) Et$_3$N, CH$_2$Cl$_2$, 4-NO$_2$C$_6$H$_4$SO$_2$Cl, 0 °C, 3 h, 95%; (b) NaN$_3$, DMF, rt, 8 h, 95%; (c) (i) H$_2$, Pd/C, rt, 2 d, (ii) HCl/Et$_2$O, (iii) NEt$_3$, 82%; (d) LiOH, 1,4-dioxane, H$_2$O; (e) Na$_2$CO$_3$, Fmoc-Cl, 1,4-dioxane, rt, 36 h, 88%.

method, completely suppressing elimination to α,β- and β,γ-unsaturated compounds. Amino ester **5** was saponified and Fmoc-protected under standard reaction conditions. The N-protected amino ester **1b** was obtained from **5** by Boc-protection with Boc$_2$O under standard conditions and was used for the crystal structure determination (Figure 1; for data, see the Supporting Information).

NH$_2$-Gly-Pro-Arg-Leu-Gly-Tyr-Ser-Trp-His-Glp

**(6)**



**7** **8**
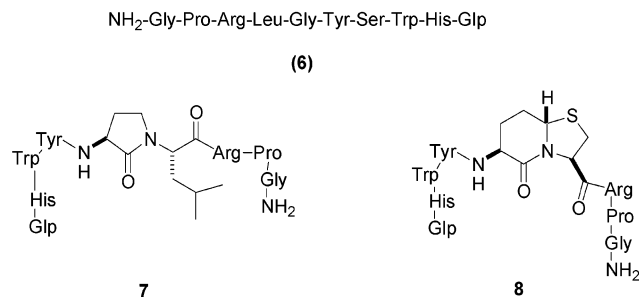
To explore the properties of **1** for generating interesting peptidomimetic structures, a target with a β-turn-containing active conformation had to be selected. In our case, the gonadotropin releasing hormone[8] [GnRH (**6**) luteinizing hormone-releasing hormone (LH-RH)] was chosen. GnRH is a decapeptide amide that is produced in the hypothalami of all mammals. Its pulsed secretion stimulates the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the anterior pituitary gland. LH and FSH are responsible for the production of sex steroids and for the gametogenesis. Several GnRH superagonists and antagonists are used for clinical treatment of endocrine-based diseases such as prostate cancer, breast cancer, and endometriosis.[9] Regarding the active conformation of **6** and its analogues, a β-turn around the central 6-position (Gly) was postulated by Monahan[10] in the early 1970s. Conformational studies by Momany,[11] based on empirical energy calculations, have been accepted as a confirmation of this assumption. The most direct way, however, to check this structure model experimentally is the synthesis of acyclic GnRH analogues in which the 6-Gly section is replaced by a
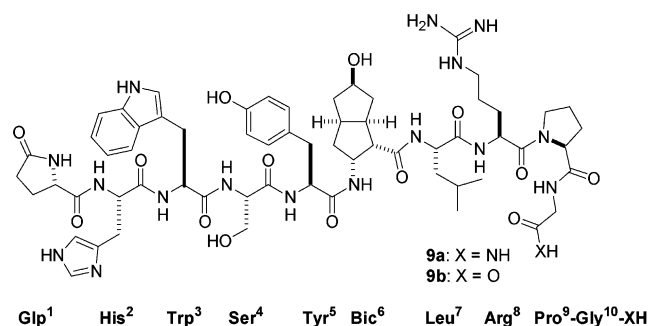


**FIGURE 2.** GnRH analogues **9a** and **9b**.

loop-inducing unnatural amino acid and the other parts of the decapeptide are left unchanged. Thus, Freidinger et al.[12] reported that GnRH derivative **7** in which the central Gly$^6$-Leu$^7$ amino acids had been replaced by a γ-lactam dipeptide induced a 8.9-fold LH release in adult ovariectomized female rats and in an in vitro pituitary cell culture compared to native GnRH. However, no detailed conformational or receptor affinity studies have been communicated. A GnRH derivative **8** with a bicyclic 5,6-dipeptide mimetic was described in ref 3a. The LH release in this case was 50% compared to native GnRH. Again, no conformational analysis was performed. The same is true of a number of other acyclic GnRH analogues, in which the postulated turn structure has been addressed by a more extensive exchange of amino acids.[8] Nevertheless, the assumption of a β-turn conformation for native GnRH is now generally accepted.

On this basis, we have incorporated amino acid **1c** into the 6-position of native GnRH to generate novel acyclic GnRH analogue **9a** (Figure 2) with a structurally defined, highly oriented turnlike conformation around position 6. In particular, the presumed conformational induction exerted by **1** with its carbocyclic framework should be different from the usual bicyclic dipeptide turn-mimetics (Figure 2), which have an amide bond in the backbone mimic.

Scheme 2 shows the solid-phase synthesis of [Bic$^6$-(OH)]-GnRH (**9a**) and [Bic$^6$(OH)]-GnRH-OH (**9b**). Both peptides were obtained by automated solid-phase peptide synthesis using a conventional Fmoc temporary protection and acid-labile resin anchors and protecting groups. Under the acidic, reductive cleavage conditions (TFA/Et$_3$-SiH/H$_2$O), the acetal on the bicyclic amino acid is removed and the resulting ketone is stereoselectively reduced to the *endo*-alcohol (Scheme 3). This OH group was left unprotected; it was envisaged as a potential anchoring group for attaching additional side chains, e.g., to form a glycopeptide.

**Conformational Analysis.** It was thought important to perform the conformational analysis under conditions close to those found physiologically. As amide **9a** is sparingly soluble in water, the free acid **9b** was chosen for the envisaged NMR study (5 mmol/L peptide in H$_2$O/D$_2$O (9:1, v/v), pH 6.5, 283 K). The assignment and analysis of the NMR spectra was done with the program ANSIG[13] resulting in distance constraints based upon 162

(8) Kutscher, B.; Bernd, M.; Beckers, T.; Polymeropulos, E. E.; Engel, J. *Angew. Chem.* **1997**, *109*, 2240−2254; *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2148−2161.
(9) Cho, N.; Harada, M.; Imaeda, T.; Imada, T.; Matsumoto, H.; Hayase, Y.; Sakasi, S.; Furuya, S.; Suzuki, N.; Okubo, S.; Ogi, K.; Endo, S.; Onda, H.; Fujino, M. *J. Med. Chem.* **1998**, *41*, 4190−4195.
(10) Monahan, M. W.; Amos, M. S.; Anderson, H. A.; Vale, V. *Biochemistry* **1973**, *12*, 4616−4620.
(11) Momany, F. A. *J. Am. Chem. Soc.* **1976**, *98*, 2990−2996.

(12) Freidinger, R. M.; Veber, D. F.; Perlow, D. S.; Brooks, J. R.; Saperstein, R. *Science* **1980**, *210*, 656−658.
(13) Kraulis, P. J. *J. Magn. Reson.* **1989**, *24*, 627−633.

**SCHEME 2.    Synthesis of Peptide Amide 9a and Peptide Acid 9b**[a]
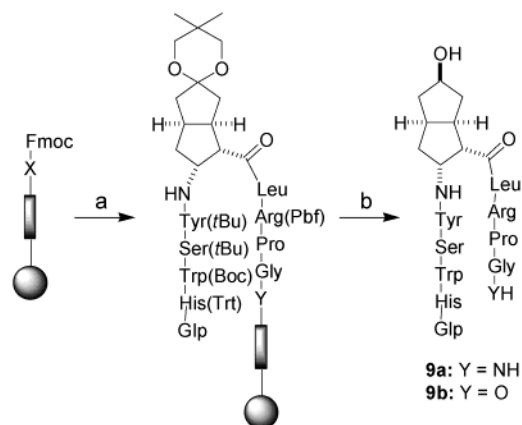


*[a]* Synthesis of **9a**: (a) TentaGel S RAM Fmoc amide resin (X = −NH−; 0.2 mmol/g), (i) Fmoc-deprotection with 20% piperidine in DMF, (ii) double coupling with 2−5.0 equiv of Fmoc-Xaa-OH or Glp-OH, using PyBOP as coupling agent and NMM as base; Xaa = Gly, Pro, Arg(Pbf), Leu, Bic (**1a**), Tyr(tBu), Ser(tBu), Trp(Boc), His(Trt); (b) final deprotection and cleavage from resin with TFA/triethylsilane/$H_2O$ = 19:1:1, 2 h, rt. Synthesis of **9b**: (a) Fmoc-Gly peptide acid resin (X = −O-Gly−), analogous to **1a** with Xaa = Pro, Arg(Pbf), Leu, Bic (**1a**), Tyr(tBu), Ser(tBu), Trp(Boc), His(Trt); (b) final deprotection and cleavage from resin with TFA/triethylsilane/$H_2O$ = 19:1:1, 2 h, rt.

**SCHEME 3.    Stereoselective Carbonyl Reduction of the Resin-Bound Peptide**
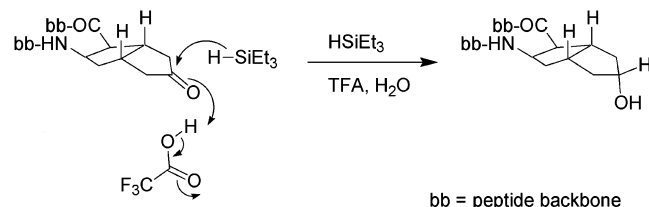


NOE contacts and a few backbone dihedral constraints based upon NH-$C_\alpha$H coupling constants (see the Supporting Information). The program XPLOR[14] was used for structure calculations starting from a linear peptide using a restrained molecular dynamics/refinement/energy minimization protocol. The resulting lowest energy structures show a partially ordered C terminus, a less ordered N terminus, and a well-defined loop region around the newly introduced amino acid (Figure 3).

As the direction of the peptide chain is reversed and no intramolecular hydrogen bond between Tyr[5] and Arg[8] is formed, this motive can be defined as a kind of an open $\beta$ turn. The distance between the $\alpha$ carbon atoms of these two residues is about 500 pm and thus lies well within the limit for a reverse turn (700 pm).[15] The experimental data show strong sequential $C_\alpha$H-NH NOEs for the 5−8 region as well as weak sequential NH-NH NOEs (see the NOE list in the Supporting Information). In addition, hydrogen exchange measurements using saturation transfer techniques in combination with gradient-selected
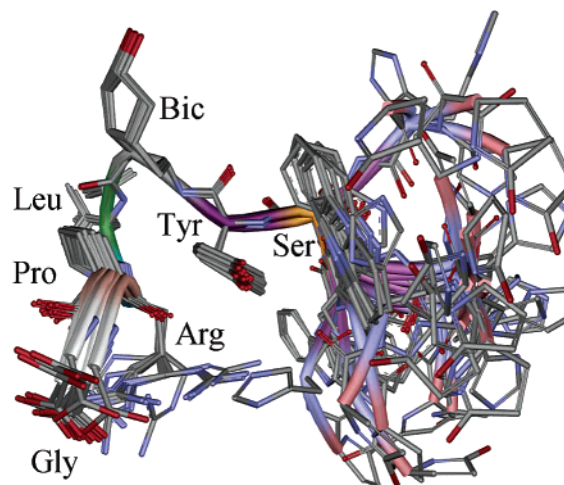


**FIGURE 3.** Superposition of 17 low energy NMR conformers of **9b** (hydrogen omitted for clarity, backbone trace through α-carbon atoms).
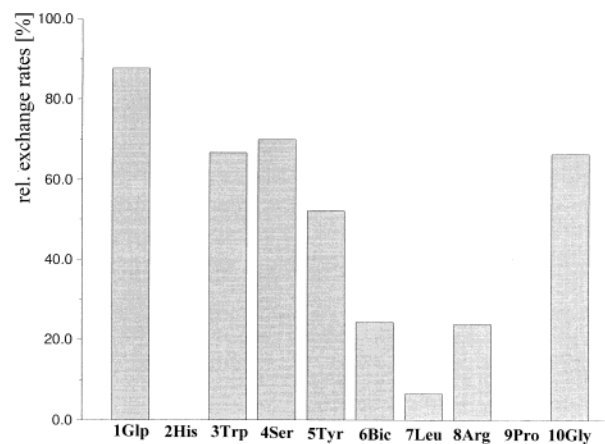


**FIGURE 4.** NH-proton exchange rates for **9b**.

$^1$H,$^{15}$N HSQC spectra show low exchange rates for the central (positions 5−8) region (Figure 4). This criterion is considered typical of amino acids within a loop conformation.[16]

To gain insight into the conformational behavior of [Bic$^6$(OH)]-GnRH (**9a**) itself, a computer model was constructed and examined using the Tinker[17] software package. Force-field parameters were taken from the OPLS-AA force field, using the generalized Born/surface area (GB/SA) water solvation model.[18] Figure 5 shows a graphical representation of the three lowest energy conformers. They represent more than 99% population of the conformational space of the peptide at room temperature.

Similar to the NMR study of **9b**, the reverse turn induced by Bic can be clearly seen in the computer model of **9a**. The distances from Ser-$C_\alpha$ to Leu-$C_\alpha$ in these three conformers are 420, 610, and 690 pm and thus lie within the limit of 700 pm for a reverse turn. In accordance with

(14) Nilges, M.; Kuszewski, J.; Brunger, A. T. In *Computational Aspects of the Study of Biological Macromolecules*; Hoch, J. C., Ed.; Plenum Press:  New York, 1991.

(15) Ball, J. B.; Hughes, R. A.; Alewood, P. F.; Andrews, P. R. *Tetrahedron* **1993**, *49*, 3467−3478 and literature cited therein.

(16) Creighton, T. E. In *Proteins−Structures and Molecular Properties*, 2nd ed.; W. H. Freeman and Co.:  New York, 1993; p 283.

(17) Kong, Y.; Ponder, J. W. *J. Chem. Phys.* **1997**, *107*, 481−492.

(18) Qiu, D.; Shenkin, P. S.; Hollinger, F. P.; Still, W. C. *J. Phys. Chem. A* **1997**, *101*, 3005−3014.
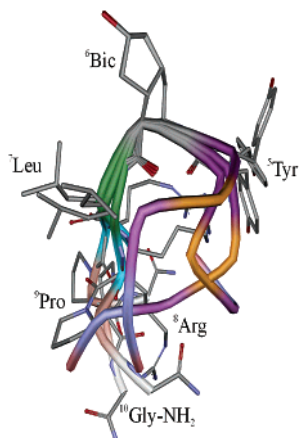
**FIGURE 5.** Superposition of three conformers of **9a** (>99% population/300 K, hydrogens and atoms of residues 1−4 omitted for clarity, backbone trace through α-carbon atoms).
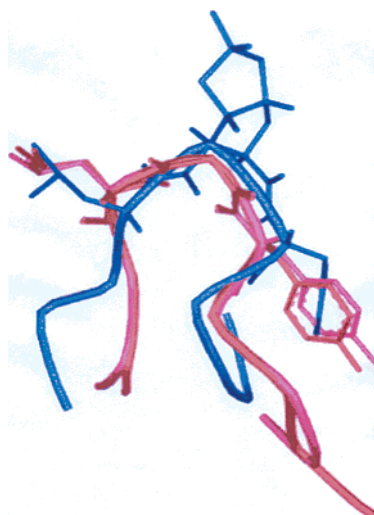


**FIGURE 6.** Superposition of one conformer of **9a** (blue) with Mamony's structure of **6** (red).

the NMR results, a relatively ordered C-terminal branch and a less ordered N terminus can be found. The structures are more compact than the NMR-derived conformers, though. The NMR-derived conformers are averaged in time by the NMR time scale and hence differ less from each other than the computationally generated ones, which are structure candidates on a molecular motion resolution. A superimposition of one of our calculated low energy structures of **9a** with the GnRH model presented by Momany shows a significant overlap in the core region (Figure 6).

A similar calculation as for **9a** was also performed for amide **9b**, and it can be seen from Figure 7 that the calculated minimum energy conformers of **9a** and **9b** are almost superimposable. This result demonstrates that, at least in the calculation, the nature of the end group is of secondary importance.

**Biological Activity.** [Bic[6](OH)]-GnRH (**9a**) was tested on the human GnRH receptor expressed in intact Chinese hamster ovary (CHO) cells. It showed a distinct affinity ($IC_{50} = 2 \times 10^{-8}$ mol/L) for the human receptor (indicated by release of IP3), which is about the same as for GnRH ($IC_{50} = 1 \times 10^{-8}$ mol/L), but less than for busereline[19]
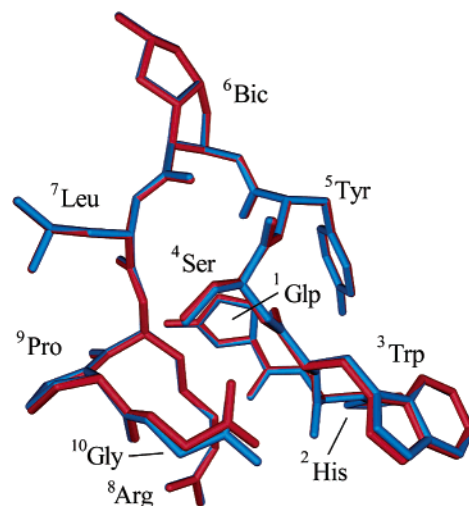


**FIGURE 7.** Superposition of the minimum energy conformers of **9a** (red) and **9b** (blue).

($IC_{50} = 1 \times 10^{-10}$ mol/L). Application in concurrency with busereline showed that **9a** is not able to completely block the receptor and thus suppress busereline-induced IP3 release. This is in accordance with its agonistic character. Compound **9a** effects $Ca^{2+}$-release in CHO#3 cells in concentrations above $10^{-8}$ mol/L. Application 4 min before application of $10^{-9}$ mol/L busereline partly inhibits busereline-induced $Ca^{2+}$-release, which can be explained by a partial antagonism of **9a** effected by de-sensitization of the GnRH receptor or its signal transduction system.

## Conclusion

In conclusion, we have shown that the novel β-amino acid Bic in the form of **1c** is suitable for inducing turn structures in oligopeptides. Thus, the GnRH analogue **9a** shows the profile of an agonist with respect to the human GnRH receptor and has an activity on a par with the natural hormone. This result is in accordance with the established assumption of a β-turn conformation of native GnRH. Additionally, the conformation of **9a** has been determined in detail by computational methods and its free acid analogue **9b** has been examined by NMR methods. Both studies independently demonstrate that a reverse turn is induced into the peptide backbone by the rigid amino acid **1c** at position 6 and that the central 5−8-section of the peptide is conformationally relatively well defined.

## Experimental Section

**General Procedures.** Reaction progress was monitored by analytical thin-layer chromatography (TLC) using silica gel 60 F$_{254}$ aluminum foils. Visualization was accomplished by UV illumination (254 nm), 0.2% ninhydrin in ethanol, anisaldehyde/sulfuric acid in ethanol (1:2:98), or phosphomolybdic acid/ceric sulfate/sulfuric acid in water (2 g/1 g/10 mL/90 mL). Flash chromatography was performed using 40−63 μm silica gel packing unless otherwise noted. ^{1}H NMR and ^{13}C NMR spectra were recorded at 250, 400, or 600 MHz. Chemical shifts (δ) are reported as parts per million from an internal tetramethylsilane (TMS) standard in the solvent indicated or by using

(19) Busereline = Glp-His-Trp-Ser-Tyr-ᴅ-Ser(tBu)-Leu-Arg-Pro-Gly-NHEt; Glp-OH = pyroglutamic acid.

the respective residual signal at 25 °C unless otherwise noted. Coupling constants were determined from ${}^1$H NMR. High-pressure liquid chromatography (HPLC) was performed using ultraviolet detection at 254 nm. For analytical reversed-phase (RP) chromatography, a 4 mm × 32 cm C-18 column was used with the solvent system indicated. Elemental analyses were performed by the Mikroanalytisches Laboratorium, Institut für Physikalische Chemie der Universität Wien. ESI spectra were recorded at the Institut für Organische Chemie der Universität Frankfurt am Main and at the Institut für Analytische Chemie der Universität Wien.

All reactions using air- or water-sensitive reagents were conducted under an Ar atmosphere with dry solvents. Solvents were purified as follows: ethyl acetate and hexanes were fractionally distilled, $CH_2Cl_2$ was distilled and filtered through alumina B (activity grade super I), DMF was distilled from $CaH_2$. Triethylamine and *N*-methylmorpholine (NMM) were refluxed for several hours over $CaH_2$ and then slowly distilled. All other reagents were purchased from commercial suppliers and used without further purification.

Automated peptide synthesis was performed on a peptide synthesizer using commercially available amide resin columns for peptide amides or columns preloaded with the first amino acid for peptide acids. Synthesis was performed using the Fmoc strategy and a 5-fold excess of protected amino acids. PyBOP was used as coupling reagent, NMM as base. Fmoc-deprotection was achieved using 20% piperidine in DMF. Final deprotection and cleavage from resin was accomplished using a cocktail of 0.35 mL of triethylsilane and 0.35 mL of distilled water in 6.3 mL of TFA. The resulting peptide amides were precipitated as TFA-salt using precooled *tert*-butyl methyl ether and separated by centrifugation.

**(1*R*,2*S*,3*S*,5*S*)-7,7-(2′,2′-Dimethyltrimethylenedioxy)-3-(4-nitrobenzylsulfonyloxy)bicyclo[3.3.0]octane-2-carboxylic Acid Methyl Ester (3).** To a solution of β-hydroxy ester **2** (56.9 g, 0.20 mol) in DCM (500 mL) at 0 °C was added triethylamine (57.0 g, 0.56 mmol, 2.8 equiv). The solution was allowed to stir for 5 min, and then 4-nitrobenzenesulfonyl chloride (62.1 g, 0.28 mol, 1.4 equiv) was added in portions during 10 min. The dark reddish brown solution was allowed to stir for 3 h at 0 °C, until complete consumption of starting material (monitored by DC analysis). A 1:1 mixture of hexanes and ethyl acetate (300 mL) was added, and the resulting suspension was filtered through a plug of silica gel. The plug was washed with hexanes/ethyl acetate (100 mL). The solvent was removed to two-thirds under reduced pressure, and the resulting suspension was again filtered. The filtrate was concentrated to a brown oil, which was dissolved in warm *tert*-butyl methyl ether (750 mL), and warm hexanes (500 mL) were added. The product was allowed to crystallize overnight, and fine yellow needles (83.3 g) were obtained. A second crystallization from the mother liquor afforded another 5.4 g product, altogether 88.7 g (95%) of **3**. [α]${}^{20}_D$ = −11.6 (*c* = 2.2, CHCl₃). ${}^1$H NMR (400 MHz, CDCl₃): δ 8.37 (ddd, *J* = 9.2, 2.2, 2.2 Hz, 2H), 8.08 (ddd, *J* = 9.1, 2.2, 2.2 Hz, 2H), 4.96 (ddd, *J* = 9.4, 9.4, 6.7 Hz, 1H), 2.92 (ψt, *J* = 8.8 Hz, 1H), 3.57 (s, 3H), 3.48 (s, 2H), 3.43 (s, 2H), 2.60−2.45 (m, 2H), 2.41−2.32 (m, 1H), 2.12−2.05 (m, 1H), 2.05−1.97 (m, 2H), 1.93−1.86 (m, 1H), 1.84−1.75 (m, 1H), 0.96 (s, 3H), 0.94 (s, 3H). ${}^{13}$C NMR (100 MHz, CDCl₃): δ 173.1, 150.7, 142.4, 129.4, 124.3, 109.4, 84.4, 72.5, 71.7, 55.0, 52.1, 42.0, 39.7, 38.5, 38.3, 36.0, 30.1, 22.5. IR (thin film): 3107 w, 2955 s, 2868 m, 1736 vs, 1608 w, 1534 vs, 1436 m, 1372 s, 1351 vs, 1313 s, 1188 vs, 1116 vs, 1094 vs, 1014 m, 964 s, 919 s, 738 vs, 685 m, 619 vs, 557 m, 512 w, 466 w cm⁻¹. MS (EI, 70 eV, 130 °C): *m/z* 469 (17.4), 410 (2.1), 368 (5.5), 267 (84.8), 207 (30.7), 181 (31.8), 128 (70.8), 93 (40.6), 69 (90.4), 41 (100). Anal. Calcd for C₂₁H₂₇NO₉S: C, 53.72; H, 5.80; N, 2.98. Found: C, 53.98; H, 5.38; N, 3.04.

**(1*R*,2*S*,3*R*,5*S*)-7,7-(2′,2′-Dimethyltrimethylenedioxy)-3-azidobicyclo[3.3.0]octane-2-carboxylic Acid Methyl Ester (4).** To a suspension of nosylate **3** (109.2 g, 0.23 mol) in DMF (150 mL) was added NaN₃ (37.7 g, 0.58 mol) at room

temperature in portions during 5 min. The dark red suspension was stirred at room temperature for 8 h, during which time it turned yellow with a colorless precipitate. Another 7.5 g (0.12 mol) of NaN₃ was added, and the suspension was allowed to stir until complete consumption of starting material according to DC analysis. Water (30 mL) was added, the resulting solution was extracted 3 × 60 mL with hexanes, and the combined organic layers were dried over MgSO₄ and concentrated to an amber residue. Chromatography on silica gel (20% ethyl acetate/hexanes) afforded a viscous oil, which was dissolved in a small amount of hexanes and cooled in a freezer to −30 °C. Crystallization was initiated by carefully warming the bottom of the flask in a warm water bath. Following this procedure, azide **4** was obtained as a slightly yellowish solid (68.1 g, 95%): [α]${}^{20}_D$ = −62.1 (*c* = 3.3, CHCl₃). ${}^1$H NMR (250 MHz, CDCl₃): δ 4.29 (ψtd, *J* = 3.8, 0.8 Hz, 1H), 3.75 (s, 3H), 3.47 (s, 4H), 2.98 (ψquint d, *J* = 9.3, 3.5, 1H), 2.91 (dd, *J* = 8.9, 4.3 Hz, 1H), 2.81 (ψquint d, *J* = 9.2, 4.1 Hz, 1H), 2.18 (ddd, *J* = 13.4, 8.4, 1.5 Hz, 1H), 2.10−1.90 (m, 3H), 1.74 (ddd, *J* = 13.5, 8.9, 4.5 Hz, 1H), 0.98 (s, 3H), 0.96 (s, 3H). ${}^{13}$C NMR (62.5 MHz, CDCl₃): δ 172.6, 110.2, 72.9, 71.9, 67.0, 56.0, 52.0, 40.7, 39.8, 39.1, 38.5, 38.1, 30.5, 22.9. IR (thin film): 2954 s, 2865 m, 2110 vs, 1740 vs 1436 m, 1330 m, 1264 m, 1106 vs, 1019 m, 881 m, 739 m, 610 vs, 513 m cm⁻¹. MS (EI, 70 eV, 60 °C): *m/z* 309 (0.8), 281 (2.3), 267 (18.2), 238 (8.6), 213 (8.2), 195 (2.5), 180 (20.5), 168 (17.0), 155 (16.5), 128 (34.4), 94 (21.3), 79 (20.2), 69 (89.3), 56 (25.8). Anal. Calcd for C₁₅H₂₃N₃O₄: C, 58.24; H, 7.49; N, 13.58. Found: C, 58.46; H, 7.35; N, 13.72.

**(1*R*,2*S*,3*R*,5*S*)-7,7-(2′,2′-Dimethyltrimethylenedioxy)-3-aminobicyclo[3.3.0]octane-2-carboxylic Acid Methyl Ester (5).** To a solution of azide **4** (30.9 g, 0.10 mol) in ethyl acetate (900 mL) was added Pd/C (10%, 350 mg), and hydrogen was allowed to bubble through the solution in a weak stream for 2 days. The solution was filtered through a plug of Celite, concentrated under vacuum, coevaporated 1 × 100 mL, 1 × 50 mL with toluene, again dissolved in diethyl ether (500 mL), and cooled to 0 °C. A solution of HCl in ether (110 mL, 1 M) was added, and the resulting hydrochloride was filtered. The solid was dissolved and partitioned between a solution of 10 g of triethylamine in 250 mL of water and 10 g of triethylamine in 250 mL of diethyl ether. The phases were separated, and the aqueous phase was again extracted 2 × 100 mL with diethyl ether. Pooled organic phases were dried (MgSO₄) and concentrated to a yellowish oil that solidified after several hours to an almost colorless solid **5** (23.2 g, 82%). ${}^1$H NMR (250 MHz, CDCl₃): δ 3.68 (m_c, 1H), 3.66 (s, 3H), 3.42 (s, 4H), 2.89 (m_c, 1H), 2.77 (ddd, *J* = 16.4, 8.2, 2.7 Hz, 1H), 2.70 (dd, *J* = 7.7, 5.1, 1H), 2.13 (ddd, *J* = 18.9, 9.0, 1.4 Hz, 1H), 2.07 (ddd, *J* = 18.7, 8.9, 1.3 Hz, 1H), 1.89−1.55 (m, 4H), 1.17 (s, 2H), 0.93 (s, 3H), 0.91 (s, 3H). ${}^{13}$C NMR (62.5 MHz, CDCl₃): δ 174.2, 109.7, 72.1, 71.8, 56.8, 55.9, 51.4, 41.8, 41.1, 39.9, 38.8, 37.8, 30.0, 22.5. IR (thin film): 3383 w, 2952 vs, 2865 s, 1730 s, 1617 br, 1473 m, 1435 s, 1203 s, 1170 s, 1115 vs, 1020 s, 907 m, 882 m, 610 vs, 511 m cm⁻¹. MS (EI, 70 eV, 60 °C): *m/z* 283 (13.2), 268 (8.6), 251 (7.0), 240 (3.2), 213 (15.3), 207 (24.4), 196 (54.5), 179 (84.3), 166 (19.5), 152 (58.9), 139 (21.3), 128 (32.3), 96 (30.6), 81 (18.7), 69 (100.0), 56 (53.4). Anal. Calcd for C₁₅H₂₅-NO₄: C, 63.58; H, 8.89; N, 4.94. Found: C, 63.52; H, 8.68; N, 4.83.

**(1*R*,2*S*,3*R*,5*S*)-7,7-(2′,2′-Dimethyltrimethylenedioxy)-3-(9*H*-fluoren-9-ylmethoxycarbonylamino)-7-oxobicyclo-[3.3.0]octane-2-carboxylic Acid (1a).** Amino ester **5** (23.24 g, 81.9 mmol) was dissolved in 1,4-dioxane (200 mL), and a solution of LiOH (6.70 g, 160 mmol) in water (200 mL) was added. The solution was allowed to stir at room temperature for 2.5 h (complete consumption of starting material). The solution was neutralized with KHSO₄ (170 mL of 1 M solution in water) and cooled to 0 °C. Na₂CO₃ was added to about 10% w/v. The solution of Fmoc-Cl (32.4 g, 125 mmol) in 1,4-dioxane (150 mL) was added under stirring within 60 min. The reaction mixture was allowed to stir at room temperature for 36 h. The solution was extracted with ether (2 × 300 mL), and the

organic phase was discarded. The aqueous phase was neutralized with 1 M KHSO$_4$ solution and extracted with ether (2 × 300 mL). The aqueous phase was acidified to pH 2−3 with KHSO$_4$ solution and again extracted with ether (2 × 300 mL). Pooled organic phases were dried (MgSO$_4$), concentrated, and chromatographed twice on flash silica gel (EtOAc/hexanes = 1:1 containing 0.5% HOAc) to afford the Fmoc-protected $\beta$-amino acid as a colorless foam (35.5 g, 88%): [$\alpha$]$^{20}_D$ = −7.5 ($c$ = 2.0, CHCl$_3$). $^1$H NMR (250 MHz, CDCl$_3$): $\delta$ 11.5−10.5 (br m, 1H), 7.75 (d, $J$ = 7.1 Hz, 2H), 7.57 (d, $J$ = 6.9 Hz, 2H), 7.35 (m$_c$, 4H), 6.89 (d, $J$ = 7.5 Hz, together 1 H), 4.56−4.14 (m, 4H), 3.49 (s, 2H), 3.46 (s, 2H), 3.00−2.62 (m, 3H), 2.37−1.94 (m, 3H), 1.84−1.53 (m, 3H), 0.98 (s, 3H), 0.96 (s, 3H). $^{13}$C NMR (carbamate cis/trans-rotamers, 62.5 MHz, CDCl$_3$): $\delta$ 178.0 and 177.4, 158.1, 155.9, 143.9, 143.7, 143.6, 141.2, 127.7, 127.1, 124.9 and 124.8, 108.9, 72.1 and 71.9, 67.5 and 66.8, 54.3 and 54.0, 53.5 and 53.0, 47.0, 42.5 and 42.1, 40.4 and 40.1, 39.5 and 39.0, 38.3, 37.2 and 37.0, 30.0, 22.5. IR (thin film): 3318 br, 2955 s, 2867 m, 1711 vs, 1522 m, 1450 m, 1333 m, 1248 m, 1227 m, 1113 vs, 1004 w, 907 m, 878 m, 759 s, 741 vs, 610 vs, 514 w cm$^{-1}$. MS (EI, 70 eV, 230 °C): $m/z$ 491 (0.3), 446 (0.3), 404 (0.5), 387 (0.1), 378 (0.1), 361 (0.3), 345 (0.1), 313 (0.2), 295 (29.0), 250 (4.9), 208 (9.0), 196 (37.8), 165 (91.0), 128 (29.4), 69 (35.8), 41 (29.0). HRMS (EI, 70 eV, 190 °C): $m/z$ 491.2321 (C$_{29}$H$_{33}$O$_6$N requires 491.2307). Anal. Calcd for C$_{29}$H$_{33}$O$_6$N: C, 70.86; H, 6.76; N, 2.85. Found: C, 70.61; H, 6.68; N, 2.58.

In an analogous way, the Boc-amino ester **1b** was prepared from **5**. $^1$H NMR (carbamate rotamers, 250 MHz, CDCl$_3$): $\delta$ 4.85 (br), 4.33 (br), 3.67 (s), 3.42 (m), 4.14 (m, 4H), 2.83 (m), 2.67 (m), 2.2 (m), 2.0 (m), 1.40 (s), 0.96 (s). $^{13}$C NMR (carbamate cis/trans-rotamers, 62.5 MHz, CDCl$_3$): $\delta$ 174.16, 155.21, 109.12, 79.29, 72.17, 71.99, 54.07, 53.59, 51.66, 42.32, 39.99, 39.12, 38.82, 37.20, 30.06, 28.34, 22.48. Anal. Calcd for C$_{20}$H$_{33}$O$_6$N: C, 62.64; H, 8.67; N, 3.65. Found: C, 62.39; H, 8.68; N, 3.58.

**Crystal Data of 1b.** A single crystal was measured on an Enraf-Nonius CAD4 diffractometer. Three standard reflections remeasured every 5500 s decreased 1% during data collection. The data were rescaled with respect to the standards. An empirical absorption correction was made on the basis of the $\psi$-scans of four reflections. The relative transmission factor ranged from 0.92 to 1.00. Equivalent reflections were averaged ($R(F)$internal = 0.016). The structure was determined by direct methods using program SIR92. A difference Fourier synthesis showed the positions of the hydrogen atoms. The H atoms were refined with isotropic thermal parameters. The non-H atoms were refined with anisotropic thermal parameters. The structure was refined on $F$ values using weighting scheme: $w(F)$ = $4F_2/[\sigma^2(F_2) + (0.03F_2)^2]$. For further data, see the Supporting Information.

**$^6$Bic(OH)-LH-RH (9a).** Solid-phase synthesis of **9a** was performed on a 0.1 mmol scale on 0.5 g of Abimed amide-resin.

Double couplings of the Fmoc-protected amino acids (2 × 5 equiv) were performed with NMM as base, PyBOP as activator, and piperidine (20% in DMF) as deprotecting agent. Histidine was protected as trityl-, tryptophane as Boc-, arginine as Pbf-derivative, and serine and tyrosine as *tert*-butyl ethers. Final capping was performed with pyroglutamic acid/PyBOP/NMM. Cleavage and deprotection with concomitant reduction of the bicycle's keto functionality to the corresponding *endo*-alcohol was accomplished using a cocktail (7 mL) of TFA/water/triethylsilane (90:5:5). The resulting peptide was precipitated with ice cold tert.-butyl methyl ether, centrifuged and purified by gel-permeation chromatography on Sephadex LH-20, using MeOH/H$_2$O = 1:1 as eluent. The peptide was further purified by RP-HPLC using MeOH/H$_2$O = 1:1 as eluent. ESI$^+$: $m/z$ 1292.1 ([MH]$^+$ = C$_{62}$H$_{86}$N$_{17}$O$_{14}$ requires 1292.6).

**$^6$Bic(OH)-LH-RH-OH (9b).** Synthesis of this compound was performed analogously to the procedure above on a 0.1 mmol scale, but instead of the amide-resin, a Fmoc-glycine pre-loaded acid resin, supplied by Abimed, was used. After cleavage/deprotection/reduction (see **9a**), the resulting solution of crude **9b** was concentrated under reduced pressure, purified by gel-permeation chromatography on Sephadex LH-20 (MeOH/H$_2$O = 1:1) and subsequent RP-HPLC (MeOH/H$_2$O = 1:1). ESI$^+$: $m/z$ 1293.0 ([MH]$^+$ = C$_{62}$H$_{85}$N$_{16}$O$_{15}$ requires 1293.6).

**NMR Structure Determination of 9b.** All measurements of a 5 mmol solution of the peptide in 90% H$_2$O/10% D$_2$O at pH 6.5 were done at 283 K on a BRUKER Avance DRX 600 spectrometer equipped with a TBI triple resonance probe and actively shielded triple axis gradient accessory. The assignment and analysis of the NMR spectra was done on Silicon Graphics workstations with the program ANSIG[13] resulting in distance contraints based upon 162 NOE contacts and a few backbone dihedral constraints based upon NH-C$_\alpha$H coupling constants. The program XPLOR[14] was used for structure calculations starting from a linear peptide using a restrained molecular dynamics/refinement/energy minimization protocol.

**Supporting Information Available:** X-ray data for **1b** and $^1$H and $^{13}$C NMR spectra including NOE and dihedral constraint lists of **9b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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